PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/16, A61K 38/16 | A1

(11) International Publication Number: **WO 98/29443**

(43) International Publication Date: 9 July 1998 (09.07.98)

(21) International Application Number: PCT/EP97/07334

(22) International Filing Date: 30 December 1997 (30.12.97)

(30) Priority Data:
9627114.3 31 December 1996 (31.12.96) GB

(71) Applicant *(for all designated States except US)*: ARMEL S.A. [LU/LU]; 50, rue Basse, L-7307 Steinsel (LU).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MABROUK, Kamel [TN/FR]; 7, boulevard Gustave Desplaces, F-13003 Marseille (FR). SABATIER, Jean-Marc [FR/FR]; La Gavotte, 12, allée des Argelas, F-13790 Châteauneuf-le-Rouge (FR). ROCHAT, Hervé [FR/FR]; 401, chemin des Saugeonnes, F-13105 Mimet (FR). VAN RIETSCHOTEN, Jurphaas [FR/FR]; Val de la Torse, Bâtiment d'Artagnan 2, F-13100 Aix-en-Provence (FR).

(74) Agent: SERJEANTS; 25 The Crescent, King Street, Leicester LE1 6RX (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MULTIPLE BRANCH PEPTIDE CONSTRUCTIONS

(57) Abstract

Multiple branch peptide constructions formed from peptides derived from the envelope transmembrane glycoprotein gp41 of HIV, and including the consensus sequence RQGY preceded by 0 to 4 amino acid residues and succeeded by 2 to 4 amino acid residues, most preferably RQGYSPL, show increased receptor affinity and prevent cell-to-cell fusion. They have a direct virostatic effect. Because they present the same peptide sequence several times, these MBPCs are able to neutralize in vitro the different steps of virus envelope/cell membrane fusion, and infected cell membrane/uninfected cell membrane fusion of several strains of HIV-1 and HIV-2. These results open a potential use in treatment of HIV infection.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

TITLE

Multiple Branch Peptide Constructions

DESCRIPTION

The invention relates to multiple branch peptide constructions (MBPCs) and to their use in the treatment of Human Immunodeficiency Virus (HIV) infections.

The use of radially branched systems in polymers has been known for a long time in classical polymer chemistry. This system has been used by J.P.Tam [*Proc. Natl. Acad. Sci. USA* 85, 5409-5413 (1988)] to develop antigens without the use of ambiguous carriers, using lysine skeletons. Those antigens were designed to generate vaccines against a variety of diseases. Antigens for generating vaccines against HIV infection are described by Tam in WO93/03766. He called them Multiple Antigenic Peptide Systems (MAPS), consistent with their conceived use. The present inventors, along with others, found that similar constructions with shorter peptides derived from the V3 loop of the surface envelope glycoprotein gp120 of HIV offered a direct therapeutic approach to the treatment of HIV infections, as described in WO95/07929. The name MAPS was then inappropriate, and the compounds were renamed as MBPCs. The MBPCs of WO95/07929 interfered with the virus envelope - cell membrane fusion step and also the infected cell membrane - uninfected cell membrane fusion step, either step being thought to be indispensable for cell infection, virus multiplication and the spread of virus in the host organism, by blockading the CD4 receptor present in cells such as lymphocytes and macrophages, apparently by attaching to a membrane co-receptor which is distinct from the CD4 binding receptor, without causing the cell to lose its ability to be activated by other antigens or mitogens.

The inventors have now discovered further MBPCs which are effective as treatments for HIV infections. These use peptides derived from the HIV envelope transmembrane glycoprotein gp41. The amino acid sequences of these MBPCs were selected on the basis of sequence homologies between various HIV isolates. The choice of gp41 amongst viral proteins was based on the following:

i) the importance of this domain in the virus-cell and cell-cell fusion processes leading to virus entry into the host cell,

ii) the importance of the gp160 splicing into gp120 and gp41 for the fusogenic activity to take place.

iii) the existence of neutralizing anti-gp41 antibodies, e.g. antibody 2F5, and

iv) the existence of a unique disulphide bridge, in contrast to gp120, which makes it easier to obtain peptides mimicking specific conformational domains of gp41.

It is presumed that the gp41-derived MBPCs of this invention interfere with a critical step of the fusion process.

The invention provides a multiple branch peptide construction and a method for the therapeutic treatment of patients with HIV infections. The multiple branch peptide construction comprises a core matrix to which are bonded from 2 to 16, and preferably from 4 to 16 peptides, each of which comprises the sequence RQGY preceded by from 0 to 4 amino acid residues and succeeded by from 2 to 4 amino acid residues. Preferably, the peptides bonded to an 8 or 16-branched core matrix are RQGYSPL. The method for the therapeutic treatment of patients with HIV infections comprises administering such an MBPC to the patient, preferably in such an amount as to induce in the patient a blood concentration of the MBPC of from 10^{-7} to 10^{-4} molar.

The core matrix is a dendritic polymer which is branched in nature, preferably with each of the branches thereof being identical. The core matrix is based on a core molecule which has at least two functional groups to which molecular branches having terminal functional groups are covalently bonded. Suitable core molecules include ammonia or ethylenediamine. Suitable molecular branches include acrylic ester monomers which are polymerized onto the core molecule. Such molecules may be created to present varying number of branches, depending on the number of monomers branched from the core molecule. The preferred core molecule is lysine. A central lysine residue is bonded to two lysine residues, each through its carboxyl group, to one of the amino groups of the central lysine residue. This provides a molecule with four amino groups, which may be the core matrix for an MBPC having four peptides. Alternatively, one can provide a molecule with eight branches by bonding four lysine residues through their carboxyl groups to one of the amino groups of the lysine residues which are attached to the central lysine. This molecule can serve as the core matrix for an MBPC having eight peptides or can alternatively receive eight lysine residues to form a core matrix for an MBPC having sixteen peptides.

The C-ends of peptides are covalently bonded to each of the branches of the core matrix to form the MBPC. The peptides may be the same, which is preferred, or may be different from one another. The resulting molecule has a cluster of peptides at the surface and an interior core matrix which is not presented and is therefore not

antigenic.

Spacers may, if desired, be included between the peptides and the core matrix. The carboxyl group of the first lysine residue may be left free, amidated, or coupled to ß-alanine or another blocking compound.

Peptides can include D or L-amino acid residues. D amino acids last longer in vivo because they are harder for peptidase to cut, but the L amino acids have better activity, as discussed below.

Moreover, peptide analogues, synthetic constructs using the carbon skeleton of peptides but omitting the -CONH- peptide bonds, can be employed in place of peptides. Thus, it should be understood that references to peptides herein may also be taken to include peptide analogues. It is believed that peptide analogues will be more resistant to peptidase and last longer in vivo.

If the peptide is too long, the MBPC will become antigenic. It is therefore desirable that each peptide should have not more than ten, and preferably not more than nine, amino acid residues.

The preferred MBPCs for use in this invention are

RL.1: $(RQGYSPL)_8$-$(K)_4$-$(K)_2$-K-ßA-OH and

RL.2: $(RQGYSPL)_{16}$-$(K)_8$-$(K)_4$-$(K)_2$-K-ßA-OH

The OH terminal shown above on the ß-alanine indicates the carboxyl group thereof, with the amino group being attached to the carboxyl group of the lysine residue. The carboxyl group of the ß-alanine may alternatively be modified to form a carboxamide terminal.

The preparation of the MBPCs of the invention, having a branched core with peptides attached thereto, can be effected by methods known in the art, see e.g. Tam et al, *J. Immun.* 148, 914-920 (1992). Preferably, for small quantities (under one kilogram), a solid phase method is used to obtain the MBPCs. Stepwise assembly of the peptide chains can be carried out automatically on 4-(oxymethyl)-phenylacetamidomethyl copoly(styrene-1% divinyl benzene). The Boc/benzyl strategy may be used, including a systematic double coupling scheme with hydroxybenzotriazole active esters (Boc-amino-acid-OBt). The final cleaving from resin is effected with strong acid, such as anhydrous hydrogen fluoride (1 hour at 0°C). The MBPC is then washed with

diethyl ether and solubilized in water. After lyophilization, the MBPC may be pre-purified on a P2 or G15 type molecular filtration column, equilibrated with 0.1N acetic acid. The eluate fraction may then be recovered. The purification step is achieved by using C_8 or C_{18} reversed-phase HPLC. The MBPC may be characterized by its amino acid content after acid hydrolysis (6N HCl, 115°C, 24 hours) and electrospray mass spectrometry.

The gp41-derived MBPCs of the invention have been tested *in vitro* for their ability to inhibit HIV-induced syncytium formation, and infection of human lymphocytes by both HIV-1 and HIV-2 viruses (several laboratory strains including LAV-2/B, an HIV-2 virus able to infect some $CD4^-$/$GalCer^-$ cells, as well as clinical isolates such as JRCSF, P16/B6 and P16/C9). The diverse peptide constructions were found to be inactive, except for MBPC RL1 which possessed potent antiviral properties in all tests. By contrast, the monomeric RQGYSPL was found to be inactive. Some results are shown in Tables 1 and 2 below. Similar results were obtained with other HIV strains and clinical isolates tested so far.

The MBPC RL1 showed neither cellular toxicity nor lethal activity when injected by the intracerebroventricular route in both C57/BL6 and Balb-C mice (concentration tested was 3×10^{-3} M, corresponding to 100 μg of peptide injected per 20g mouse.

TABLE 1

Inhibition of the Hx10 (HIV-1) strain infectivity by the MBPCs RL1 and SPC3

Peptides	Molarity	OD	p24 (ng/ml)	Inhibition (%)
SPC3-D4	5×10^{-5}	0.052	0.05556143	98.89
	1×10^{-5}	0.211	0.48654334	89.13
	5×10^{-6}	0.849	2.21589212	50.78
	1×10^{-6}	1.797	4.78552009	0
SPC3-D5	5×10^{-5}	0.066	0.09350952	98.47
RL1-D4	5×10^{-5}	0.047	0.04200854	99.09
	1×10^{-5}	0.359	0.88770888	79.82
	5×10^{-6}	0.657	1.69546114	61.45
	1×10^{-6}	1.148	3.02635495	31.29
RL1-D5	5×10^{-5}	0.035	0.00948160	99.75

SPC3 is $(GPGRAF)_8$-$(K)_4$-$(K)_2$-K-ßA-OH as disclosed in WO95/07929.

D4 and D5 refer to days 4 and 5 of the experiment.

OD stands for Optical Density.

N.B. Experiments were performed with non diluted virus solution.

TABLE 2

Inhibition (%) of clinical isolates infectivity by the MBPCs RL1 and SPC3.

[conc]	1×10^{-5}	5×10^{-6}	1×10^{-6}	5×10^{-7}	1×10^{-7}	5×10^{-8}
SPC3	85.7	50.0	28.6	0	0	0
RL1	89.3	70.0	67.1	40.8	18.3	0

Example shown is the HIV-1 W5A2A9 isolate.

N.B. Experiments were performed with non diluted virus solution.

CLAIMS

1. A multiple branch peptide construction comprising a core matrix to which are bonded from 2 to 16 peptides, each of which comprises the amino acid sequence RQGY preceded by 0 to 4 amino acid residues and succeeded by from 2 to 4 amino acid residues.

2. A peptide construction according to claim 1 in which there are from 4 to 16 peptides bonded to the core matrix.

3. A peptide construction according to claim 1 or claim 2 in which each peptide is the same.

4. A peptide construction according to claim 1 or claim 2 in which each peptide is RQGYSPL.

5. A peptide construction according to claim 4 in which there are 8 or 16 peptides RQGYSPL.

6. A peptide construction according to any of claims 1 to 3 in which each peptide includes not more than 10 amino acid residues.

7. A peptide construction according to any of claims 1 to 3 in which each peptide includes not more than 9 amino acid residues.

9. A peptide construction according to any preceding claim in which the core matrix is comprised of lysine residues.

10. A peptide construction according to any preceding claim in which there are spacers between the core matrix and the peptides.

11. A peptide construction according to claim 1 in which the peptides are peptide analogues.

12. A peptide construction according to claim 1 in which the peptides include at least one D-amino acid residue.

13. A peptide construction according to any preceding claim which is non-immunogenic at a blood concentration of up to 10^{-4} molar.

14. A medicament comprising a multiple branch peptide construction according to any preceding claim in admixture with a pharmaceutically acceptable diluent or carrier.

15. Use of a multiple branch peptide construction according to any of claim 1 to 13 for the preparation of a medicament for the treatment of HIV infections.

16. A method of treating HIV infections comprising administering to a patient a multiple branch peptide construction according to any of claims 1 to 13.

17. A method according to claim 16 in which the multiple branch peptide construction is administered to the patient in an amount sufficient to induce in the patient a blood concentration of less than 10^{-4} molar.

18. A method according to claim 16 or claim 17 in which the multiple branch peptide construction is administered to the patient in an amount sufficient to induce in the patient a blood concentration of more than 10^{-7} molar.

19. A method of treating HIV infections comprising administering to a patient a medicament according to claim 14.

20. A method according to claim 19 in which the medicament is administered to the patient in an amount sufficient to induce in the patient a blood concentration of the multiple branch peptide construction of less than 10^{-7} molar.

21. A method according to claim 19 in which the medicament is administered to the patient in an amount sufficient to induce in the patient a blood concentration of the multiple branch peptide construction of more than 10^{-4} molar.

22. A method for the preparation of a multiple branch peptide construction comprising a core matrix to which are bonded from 2 to 16 peptides, each of which comprises the amino acid sequence RQGY preceded by 0 to 4 amino acid residues and succeeded by 2 to 4 amino acid residues, the method comprising solid phase stepwise elongation of the peptide chains on a resin, followed by cleavage of the multiple branch peptide construction from the resin.

23. A method according to claim 22 in which the resin is 4-oxymethyl-phenylacetamidomethyl copoly(styrene-1% divinylbenzene).

24. A method according to claim 22 or claim 23 in which the Boc/benzyl strategy is used, including systematic double coupling with hydroxybenzotriazole active esters (Boc-amino-acid-OBt).

25. A method according to any of claims 16 to 18 in which the cleavage from the resin is effected with anhydrous hydrogen fluoride at 0°C.

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/EP 97/07334

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/16 A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 07929 A (ARMEL SA ;MCKELVEY IAN EDWARD (GB)) 23 March 1995 cited in the application	1-25
X	see claims 16-19	22-25

A	WO 93 03766 A (REPLIGEN CORP ;UNIV ROCKEFELLER (US)) 4 March 1993 cited in the application see the whole document	

A	N. YAHI ET AL.: "Multibranched V3 peptides inhibit human immunodeficiency virus infection in human lymphocytes and macrophages" J. VIROLOGY, vol. 68, no. 9, September 1994, pages 5714-5720, XP002063190 see the whole document	

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 1998	20. 05. 1998
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	**Authorized officer** Cervigni, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 97/07334

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RATNER L ET AL: "COMPLETE NUCLEOTIDE SEQUENCE OF THE AIDS VIRUS, HTLV-III" NATURE, vol. 313, 24 January 1985, pages 277-284, XP000572709 *see aa. residues 715-722 coded by env gene* see the whole document see page 279, column 2 -----	

1

INTERNATIONAL SEARCH REPORT

In. ational application No.
PCT/EP 97/07334

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 16-21 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter nal Application No
PCT/EP 97/07334

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9507929 A	23-03-95	AP 502 A	07-06-96
		AU 7619694 A	03-04-95
		BR 9407530 A	26-08-97
		CA 2171531 A	23-03-95
		CN 1130911 A	11-09-96
		EP 0719281 A	03-07-96
		HU 75092 A	28-04-97
		JP 9504273 T	28-04-97
		PL 313520 A	08-07-96
		US 5622933 A	22-04-97
		ZA 9407053 A	02-05-95
WO 9303766 A	04-03-93	NONE	